REMARKS

Claims 119-138 are presented for consideration.

As an initial matter, the cover page of the Office action is marked incorrectly by indicating that certified copies of the priority documents were not received. The priority documents were received in the parent national stage (§371) application from the International Bureau, as shown on the Notification of aAcceptance mailed in the parent application (copy attached).

In response to the Office action and with respect to the prior art of record, applicants submit hereby a new set of claims, in which the main claim is a combination of claims 85 and 88 taking into consideration the claim rejections under second paragraph of 35 U.S.C. 112. The subject of preferred embodiments of former claim 85 can be found in the dependent claims. The wording "object plane" has been replaced throughout the claims by the wording "image plane". For a person skilled in the art this is immediately apparent from looking e.g. at figures 10 and 14 of the application, where a pinhole aperture is shown in the image plane of the device (and not in the object plane where the sample is situated) or looking e.g. at figure 1 and the corresponding text on page 164, right column, line 9 of Kask et al.

As described in the the instant specification, applicants submit an additional independent claim 120, featuring the substitution of the confocal pinhole by a confocal detector device. This instrumental set-up is disclosed, e.g., on page 72 of the specification "photodiodes ... can replace the pinhole apertures in terms of a confocal detector . . . instead of the pinhole apertures, one or more semiconductor detector elements for example can be placed in the image plane."

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Former claim 87 has been split into two separate claims 125 and 130. Former claim 94 has been split into four separate claims 132 to 135. To address issues raised in the § 112 rejections, previously of record regarding former claim 96, claims 137 to 138 have been drafted.

Reconsideration of the rejections under 35 USC §§ 102 and 103 is respectfully requested in that the claims presented, hereby, are neither anticipated by, nor obvious over, the prior art relied on in the statements of rejection.

For anticipation to exist each and every claim limitation, as arranged in the claim, must be found in a single prior art reference. *Jamesbury Corp. v. Litton Industrial Products, Inc.*, 225 USPQ 253 (Fed. Cir. 1985). The absence from a prior art reference of a single claim limitation negates anticipation. *Kolster Speedsteel A B v. Crucible Inc.*, 230 USPQ 81 (Fed. Cir. 1986). A reference that discloses "substantially the same invention" is not an anticipation. *Jamesbury Corp.* To anticipate the claim, each claim limitation must "*identically* appear" in the reference disclosure. *Gechter v. Davidson*, 43 USPQ2d 1030, 1032 (Fed. Cir. 1997) (*emphasis added*). To be novelty defeating, a reference must put the public in possession of the identical invention claimed. *In re Donahue*, 226 USPQ 619 (Fed. Cir. 1985).

Rigler et al. disclose a FCS apparatus wherein the apparatus comprises a laser beam generator, a focussing lens, a fluorescence detector, and a pinhole aperture.

Kask et al. disclose a microfluorimeter which was primarily designed for FCS in the milli- and microsecond time range. The beam from a cw argon ion laser is focussed into a microcuvette using a lens and an objective. An aperture is positioned in the image plane of the observation objective. The light emerging from the aperture is divided by a beamsplitter for observation by two photon detectors.

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Sorscher et al. disclose also a FCS apparatus. An argon laser provides illumination thereby exciting fluorescence in a sample. Fluorescence is collected by the microscope objective lens, and directed toward a photomultiplier. An aperture restricts the field of view to the center of the sample.

Thompson et al. disclose an optical apparatus of TIR/FCS wherein the apparatus comprises an argon laser to illuminate the sample. Fluorescence originating forom the sample is collected by the microscope objective and filtered. The filtered light is directed through an image plane aperture to the photomultiplier.

Meyer al. disclose a FPC apparatus comprising an argon ion laser, a specifically constructed objective of a working distance of 14 mm and a single photon multiplier. Unwanted fluorescence is rejected by an adjustable diaphragm in the image plane.

Hirschfeld et al. disclose an apparatus for detecting and classifying nucleic acid particles. The apparatus comprises a radiation source, means for directing illumination into a specimen and detection means for viewing fluorescence emission. The document does not disclose to arrange a pinhole aperture in an image plane within a beam path of fluorescence radiation confocally with respect to the measuring volume.

Neither Rigler et al., Kask et al., Sorscher et al., Thompson et al., Meyer et al., nor Hirschfeld et al. teach placing the detector into the image plane or arranging the measuring volume by not more than 1,000 μm from the focusing device. Since each and every claim limitation of claims 119 and 120 is not found in any one of the cited references, these claims and all dependant claims are not anticipated under §102, and, the rejections under § 102 are in order to be withdrawn.

The presently claimed invention is not obvious under §103 because: (i) the results of the invention are substantially superior to results achieved applying state-of-the-art methods; (ii) critical

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limitations (distance of ≤ 1000 μm) are necessary for the success of the invention; (iii) the invention, based on its merits, is successful commercially. The presently claimed invention does not merely substitute equivalents for prior art devices but, rather, the change to a distance of ≤ 1000 μm effects disproportionate and unexpected results.

Attached as Appendix are test experiments performed and their results. As the "objective" disclosed in Meyer et al. is not commercially available, the tests substitute commercially available objective with a distance to the measuring volume which is significantly distinct from the distance taught by the invention.

Tests performed compare arranging the measured volume at a distance of > 1000 μm from the laser focussing optic" employed in the cited reference with arranging "the measured volume at a distance of ≤ 1000 μm from the laser focussing optic" employed in the invention described and claimed in the above-identified patent application.

In accordance with the invention, different samples (Appendix) were exposed to a laser beam. The focus of the laser beam, i.e. the measured volume, was arranged at a distance of ≤ 1000 μm from a laser focussing optic. The laser focussing optic was an objective Olympus Plan Apo 40 x 0.9 W LS with a distance to the measuring volume of 420 μm. Fluorescence was excited in this sample and measured at measuring times ≤ 500 ms using detection optics. Material-specific parameters such as "counts per molecule (cpm)", "their standard deviation (STD cpm)", "diffusion times (Diff)", "their standard deviations (STD Diff.)" and "particle number (n)" were determined by Fluorescence Correlation Spectroscopy. The parameter "counts per molecule" is a measure for the number of detected photons per time unit which have been emitted by a fluorescent substance in the focus.

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To compare this result with a method wherein the measured volume is arranged at a distance of > 1000 μm from the laser focussing optic disclosed in the prior art, the procedure described in the preceding paragraph was repeated with arranging the measured volume at a distance of 3300 μm from a laser focussing optic instead of 420 μm. Therefore, an objective Olympus LUMPlan FI 40 x 0.8 W with a distance to the sensed volume of 3300 μm was used.

The results of this test are summarized in Figures 1 and 2 as well as in Tables 1 and 2 (Appendix).

The higher the count number per molecule, the brighter the molecule or the more efficient the detecting optic. The test on the samples "TAMRA" and "TAMRA labelled peptide*" shows that by arranging a measured volume at a distance ≤ 1000 μm the count number per molecule is as twice as high as by arranging a measured volume according to the prior art. This factor 2 in count rate is surprising because the objectives differ due to their numeric aperture with regard to their photon collection efficiency only by a factor 1.3.

Tests have been performed on samples comprising additives which influence the optical properties in a way which occurs frequently in biological material. In a first instance, an additional optical absorption has been generated by the addition of ink. In another instance, an increase in the sample's index of refraction has been generated by the addition of sucrose. A person skilled in the art would expect that with increasing index of refraction both objectives create equally increased sizes of the confocal volumes and accordingly – due to the decreased laser power density in the confocal volumes - decreased count rates per molecule. Surprisingly, the measurement according to the invention is influenced only marginal, whereas the count rate per molecule is strongly decreased applying the prior art teaching.

The signal-to-noise-ratio, defined as measured value / standard deviation of measured value, has been determined (see Fig. 2). Surprisingly, the signal-to-noise-ratio is significantly better arranging the measured volume at a distance of ≤ 1000 μm from the laser focussing optic according to the claimed invention in comparison to the prior art.

The above tests demonstrate clearly the superiority and criticality of arranging a measured volume at a distance of ≤ 1000 μm from a laser focussing optic as presently claimed. The aforementioned superiority and criticality with respect to arranging a measured volume at a distance of ≤ 1000 μm from a laser focussing optic according to the presently claimed invention would have been unobvious to one skilled in the art.

Favorable action is requested.

Respectfully submitted,

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